

## Detection of Aflatoxin B1 in Buffalo Feed Samples from Cities of Ahvaz and Karun

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**ABSTRACT**

**Background and Objective:** Mycotoxins are a group of relatively resistant toxic metabolites. The most important mycotoxins are aflatoxins (B1 and B2, G1 and G2), which originate from contaminated animal feed. Dairy cattle transmit aflatoxins B1 and B2 through milk in form of aflatoxins M1 and M2, and endanger the human health. Traditional buffalo farms play an important role in the supply of dairy products in Khuzestan Province. In addition, the province has suitable conditions for the growth of various types of fungi. Therefore, this study aimed to determine the amount of aflatoxins in buffalo feed samples collected from two main suppliers of milk in the province (Ahvaz and Karun).

**Methods:** Overall, 60 samples were collected during the 3 months of autumn 2014. Samples were analyzed by the sensitive and fast method of competitive enzyme-linked immunosorbent assay.

**Results:** The concentration of aflatoxin ranged from 0.77 to 64.85  $\mu\text{g}/\text{Kg}$ . In addition, the concentration of aflatoxin in 21 samples was higher than the permitted limit (25  $\mu\text{g}/\text{Kg}$ ).

**Conclusion:** The mean concentration of aflatoxin in the samples increases with the decrease in temperature and humidity. This increase is observed in Ahvaz at a higher rate, which could be due to inappropriate storage and use of rice bran in their feed.

**Keywords:** Aflatoxins, Ahvaz, Animal Feed.

## INTRODUCTION

Mycotoxins are a group of relatively resistant toxic metabolites produced by fungi in their secondary metabolism, causing environmental contamination (1). Many mycotoxin-producing fungi grow well in hot and humid climates. Aflatoxins are the most important type of mycotoxins, which are categorized into types B1, B2, G1 and G2. These aflatoxins are produced by the *Aspergillus* genus, especially *Aspergillus flavus* and *Aspergillus parasiticus* and *Aspergillus nomius*. These species are spread throughout the world, and can cause food contamination (2,3). These toxins could be found in human foods and animal feeds. Aflatoxin B1 is the most toxic form of aflatoxins (4). Aflatoxins M1 and M2 are the metabolites of aflatoxins B1 and B2 in humans (5). These aflatoxins are found in dairy products from livestock that have been exposed to aflatoxin-contaminated feed (2,6-8). When animals eat aflatoxin B1-contaminated feed, they convert the toxin into aflatoxin M1 in their milk (9, 10). Aflatoxin M1 can resist thermal changes such as pasteurization, sterilization, autoclaving and other processes, without any reduction in its concentration (11-14). In 1998, the Food and Agriculture Organization investigated the level of livestock feed contamination in 30 countries (15). The highest level of aflatoxin contamination was found in hot and humid countries such as India (16), Indonesia (17) and Nigeria (18) with a concentration range of 1-6000 µg/Kg. The concentration of aflatoxins was reported to be between 1-400 ppb in a study on 709 livestock feed samples from the state of Kerala, India. Aflatoxin B1 contributed to 66-82% of total aflatoxins. The mentioned study also reported that the level of aflatoxins increased significantly during the rainy season (19). In a study by the US Food and Drug Administration on aflatoxins in 2014, 1.6% of corn samples and 6.6% of cotton seed samples were contaminated with toxins, with aflatoxin concentrations higher than 300 ppb (20). Study of Faraji et al. on level of aflatoxins in rice used in Mashhad during summer and winter showed that the mean level of aflatoxins B1 and B2 was 2.55 and 0.34 µg/Kg, respectively, while the mean level of aflatoxins G1 And G was negligible. µg/Kg in the samples collected in October. In November, the mean concentration of

In 3.8% of the samples, the level of aflatoxin B1 was higher than the regulatory limit set by the Institute of Standards and Industrial Research of Iran. Moreover, the mean amount of aflatoxins was higher in the samples collected in summer compared to those collected in winter (21). In study of Ersali et al. in Shiraz, 46.43% of animal feed samples contained aflatoxin B1 levels higher than the regulatory limit. The amount of contamination in summer and autumn was more than that in winter and spring (22). In study of Mehraban Sang Atash et al. in Khorasan Razavi Province, the level of aflatoxin B1 in all food items was higher in winter than in summer. In addition, 21.87% of the samples had aflatoxin B1 contamination level higher than the standard limit (23). In study of Maktabi et al. on 88 samples from cattle feed of traditional dairy farms in Ahvaz, level of aflatoxin B1 in five samples (5.68%) was higher than the standard limit. Concentrate and bagasse of sugarcane are the main contributors to contamination with aflatoxin B1 in summer, while rice bran is the main source of contamination in winter (24). Khuzestan is the country's number one region for breeding buffalos, and buffalo farms are important supplier of dairy products for the province. Therefore, we investigated the level of aflatoxins (B1, B2, G1, G2) in buffalo feed samples.

## MATERIAL AND METHODS

Samples were collected during October-December 2014 from cattle farms in the regions of Gavmish Abad and Mashali according to the latest statistics of the Ministry of Agriculture Jihad of Khuzestan Province (2008). Enzyme-linked immunosorbent assay (ELISA) was used to assess aflatoxin levels due to its high accuracy and quick analysis time. Aflatoxin B1 was measured using EuroProxima kits (5121AFT1p [2]03.09., Netherland). At least 200 g was taken randomly from different parts of the feed containers, and stored in plastic and paper bags in cold and dry conditions, away from sunlight. The samples were transferred to the laboratory and stored in a freezer at -20 °C (25, 26). Overall, 60 animal feed samples were collected with 95% confidence interval. After the samples were removed from the freezer at same conditions, they were dried

powdered samples was mixed with 9 ml of 80% methanol. The mixture was placed on a shaker for 30 min at room temperature.

After centrifuging the mixture at 2000g for 10 min, 50  $\mu$ l of the supernatant was taken and mixed with 150  $\mu$ l of buffer solution. A 20% methanolic mixture was prepared. After washing and adding the standards into the wells, 50  $\mu$ l of the solution was added to the ELISA microplate. Level of aflatoxin B1 was measured after preparing the reagents according to the manufacturer's instructions.

## RESULTS

Of the 60 samples, 21 contained aflatoxin concentrations above the Iranian National Standard limit (25  $\mu$ g/kg). The mean concentration of aflatoxins was 23.61  $\mu$ g/Kg (concentration range: 0.77-64.85  $\mu$ g/kg).

In October, the mean concentration of aflatoxins in the samples collected from Gavmish Abad and Mashali was 9.08  $\mu$ g/Kg and 16.8  $\mu$ g/Kg, respectively. Moreover, the mean concentration of aflatoxins was 12.94

aflatoxins in the samples collected from Gavmish Abad and Mashali was 25.8  $\mu$ g/Kg and 26.31  $\mu$ g/Kg, respectively. The mean concentration of aflatoxins in the samples collected in November was 36.05  $\mu$ g/Kg. In December, the mean concentration of aflatoxins in samples from Gavmish Abad and Mashali was 28.21  $\mu$ g/Kg and 35.47  $\mu$ g/Kg, respectively. The mean concentration of aflatoxins in samples collected in December was 31.84  $\mu$ g/Kg. During the three consecutive months, the mean concentration of aflatoxins in the samples collected from Gavmish Abad and Mashali was 21.03  $\mu$ g/Kg and 26.19  $\mu$ g/Kg, respectively. The mean concentration of aflatoxins in the two regions during the three consecutive months was 23.61  $\mu$ g/Kg (Table 1). There was a significant difference between the mean concentration of aflatoxins in the two regions ( $p= 0.032$ ). In addition, the mean concentrations of aflatoxins in October and November differed significantly ( $P = 0.00$ ). The mean concentrations of aflatoxins in October and December also differed significantly ( $P=0.04$ ).

Table 1- Concentrations of aflatoxins in samples collected from Gavmish Abad and Mashali

City of Karun (Gavmish Abad)					City of Ahvaz (Mashali)					Month
Standard deviation	Mean level of contamination ( $\mu$ g/Kg)	Contamination range ( $\mu$ g/Kg)	Number of contaminated samples exceeding the permissible limit	Number of Samples	Standard deviation	Mean level of contamination ( $\mu$ g/Kg)	Contamination range ( $\mu$ g/Kg)	Number of contaminated samples exceeding the permissible limit	Number of Samples	
7.12	9.08	0.77-26.55	1	10	3.51	16.80	13.36-23.48	-	10	October
3.64	25.80	34.94-20.75	4	10	8.70	26.31	8.83 – 57.79	4	10	November
9.05	28.21	47.69 – 18.95	5	10	16.16	35.47	16.95-64.85	7	10	December

## DISCUSSION

The origin of all aflatoxins in animals is contaminated feed. Lactating animals transmit aflatoxins B1 and B2 through milk in the form of aflatoxins M1 and M2, and endanger human health. Therefore, it is essential to prevent and monitor this issue. Traditional buffalo farms play an important role in the supply of dairy products in the Khuzestan Province. In addition, the climate conditions of this province are suitable for the growth of different types of fungi. We studied two regions of Gavmish Abad and Mashali that are ranked first in number of buffaloes and buffalo milk production in the province. In this regard, Gavmish Abad. This could be attributed to the difference in the type of feed used and method of feed storage in the two regions. Mixture of

the three months of autumn in Iran (October-December) were investigated due to the long interval until harvest and the suitable humidity and temperature for the production of fungi (27).

In a study in Qom, the amount of aflatoxins was evaluated in 40 forage samples. The mean amount of aflatoxins was reported to be 1.83  $\mu$ g/Kg, but the amount of aflatoxins in the samples was less than the maximum permitted limit in Iran and Europe (28). A study in Tabriz evaluated the amount of aflatoxins in forage samples. The toxin was detected in all samples with concentrations ranging from 1 to over a 4-hour period. Then, the samples were milled and powdered. According to the kits manufacturer's instructions, 3 g of the

40 µg/Kg, and mean concentration of 5.5 µg/Kg. In addition, 27% of the samples had aflatoxin contamination levels higher than the maximum permitted limit in most countries (29). Study of Fatemeh Zaboli et al. reported that almost all collected samples contained aflatoxin, and the mean amount of aflatoxin B1 in old samples (with shelf-life) and new samples (without shelf-life) was 3.43 ng/g and 3.53 ng/g, respectively.

There was a significant difference between the new and the old samples. The results of the mentioned study demonstrated the presence of aflatoxin contamination in most rice samples, but its level was lower than the national standard limit in Iran (30). Several factors such as temperature and humidity of the environment, food composition, pH, food storage, damage incurred at the time of harvest, and maintenance of agricultural products affect the growth of fungi and aflatoxin production in animal feed (31-34). Therefore, the concentration and level of contamination of livestock feed varies in different geographical locations and different types of dairy farms.

In the present study, there was a significant difference in the mean concentration of aflatoxin between the three months ( $P = 0.00$ ), while there was no significant difference between the mean concentration of aflatoxin in October and December ( $P = 0.119$ ).

The highest concentration of the toxin was recorded in Mashali and in December (64.85 µg/Kg), while the lowest concentration of the toxin was recorded in Gavmish Abad and in October (0.77 µg/Kg).

Moreover, the mean concentration of aflatoxins was higher in colder months. This could be due to increased level of moisture (due to rainfall) and the suitable temperature

for growth of fungi. The mean rate of aflatoxin production in Mashali was higher than that in bagasse, straw and rice bran was used in Mashali, while mixture of bagasse and wheat which is considered an important source of aflatoxin contamination, the mean concentration of aflatoxin was higher in this bran was used in Gavmish Abad. Since the feed used in Mashali contained rice bran region. In Gavmish Abad, feed is kept in plastic bags and in a storehouse while on the ground, which could provide suitable conditions for the growth of toxin-producing fungi.

## CONCLUSION

Monitoring and controlling the level of aflatoxin contamination are of great importance in all seasons, especially in autumn and summer (due to high humidity in autumn and high temperature in summer). In addition, rice bran in livestock feed should be replaced with an alternative that has lower risk of contamination (such as wheat flour, sunflower meal, etc.)

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## CONFLICT OF INTEREST

All contributing authors declare no conflicts of interest.

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